AMENDMENTS TO THE SPECIFICATION:

Please amend the specification as shown:

Please delete the paragraph on page 4, lines 24-30 and replace it with the following paragraph:

Figure 4 (**Residues 1-779 of** SEQ ID NO~~S~~ 1 and **SEQ ID NO:** 4) describes the protein sequence described by the DNA sequence coding for apopholasin and shows, in Figure 4A, the complete sequence (SEQ ID Nos 1 and 4) of the positive clone 40 identified from the *Pholas dactylus* light organ library. The first 20 amino acids at the N-terminus are a signal peptide, and this can either be retained or removed when generating the BIOP as described in this invention and, in Figure 4B (SEQ ID NO: 1), the cDNA coding for apopholasin with untranslated 5′ and 3′ ends. The untranslated regions are also shown;

Please delete the paragraphs on page 5, lines 1-12 and replace it with the following paragraphs:

Figure 6 (SEQ ID NO: 1, ~~piece~~ **Residues 31-848** of SEQ ID NO: 1 and SEQ ID NO: 23) shows the sequence for apopholasin genomic DNA. Two gDNA clones were indentifed but no introns were found; the Figure shows an alignment of the cDNA from cDNA clone 40 (SEQ ID NO: 1) and the gDNA amplified by both r*Tth* DNA polymerase XL (SEQ ID NO: 23) and BioXAct polymerase (~~piece~~ **Residues 31-848** of SEQ ID NO: 1). The sequences of the PCR product and the inserts in pGEM T were aligned with the sequence of the cDNA of clone 40 and were identical to this cDNA;

Figure 7 (SEQ ID NOS 7-22) describes the oligonucleotides used for screening and expression. Degenerate oligonucleotides for

library screening are shown in Figure 7A; (SEQ ID NOS 7-10) non-degenerate ones in Figure 7B (SEQ ID NOS ~~7~~ **11**-16); and oligonucleotides used for protein expression are shown in figure 7C (SEQ ID NOS 17-22).

Please delete the paragraph on page 5, lines 14-15 and replace it with the following paragraph:

Figure 9 (~~piece~~ **Residues 30-779** of SEQ ID NO: 1) is a schematic representation of Figure 8 mapped to the sequence of Figure 4A (translated region), and

Please delete the paragraph on page 5, lines 18-24 and replace it with the following paragraph:

Accordingly, the present invention provides recombinant DNA encoding the apophotoprotein apopholasin and comprising the nucleotide sequence of the sequence disclosed in Figure 4B (SEQ ID NO: 1). Three different cDNAs coding for apopholasin have been isolated, having differing non-coding regions, respectively disclosed in Figure 1 (SEQ ID NOS 1-3). The genomic DNA (gDNA), which contains no introns, has been shown (Figure 6) (SEQ ID NO: 1, ~~piece~~ **Residues 31-848** of SEQ ID NO: 1, SEQ ID NO: 23) to comprise the same basic sequence as the cDNA.

Please delete Table 1 on page 6 replace it with the following table:

Protein	Homologous region cloned protein homology (+ denotes a conserved amino acid) selected protein

tRNA-splicing endonuclease β subunit (~~piece~~ **Residues 158-199** of SEQ ID NO: 4) *Saccharomyces cerevisiae* EC 3.1.27.9 (SEQ ID NO: 24)	SLYDEDNNGVMDEGKVIPSETIE +L DEDNN + + G ++P E++E NLRDEDNNLLDENGDLLPLESLE LDQDVELDYTW LD DV DYTW LDHDVSKDYTW
ATP-AMP transphosphorylase (~~piece~~ **Residues 147-177** of SEQ ID NO: 5) *Cyprinus carpio* EC 2.7.4.3 (SEQ ID NO: 25)	VMDEGKVIPSETIEDDIKDCGLLDQDVELD Y +M +G+++P +T+ D IKD + DV Y IMQKGELVPLDTVLDMIKDAMIAKADVSKG Y
DNA primase (~~piece~~ **Residues 21-40** of SEQ ID NO: 6) *Synechocystis sp.* EC 2.7.7. -(SEQ ID NO: 26)	EEVQCAMNWTQANEYVFNVD ++VQ M ++Q+ + +FN D DQVQSLMRFSQSKQIIFNFD
purine permease (~~piece~~ **Residues 23-36** of SEQ ID NO: 6) *Emericella nidulans* (SEQ ID NO: 27)	VQCAMNWTQANEYV + C+++WT+ N ++ IMCSVDWTRRNRFI
DNA repair protein complementing XP-A cells homologue (~~piece~~ **Residues 187-200** of SEQ ID NO: 5) *Drosophila melanogaster* (SEQ ID NO: 28)	PDTVDEAEDTPSET PDT DE EDT + T PDTYDEEEDTYTHT
ATP synthase β chain (~~piece~~ **Residues 188-200**of SEQ ID NO: 5) *Peptococcus niger* EC 3.6.1.34 (SEQ ID NO: 29)	DTVDEAEDTPSET D +DEA + PSET DPIDEAGEVPSET
DNA polymerase α (~~piece~~ **Residues 161-185** of SEQ ID NO: 6) *Homo sapiens* EC 2.7.7.7 (SEQ ID NO: 30)	DEDNNGVMDEGKVIPSETIEDDIKD D+D G +++G+ I + +EDD D DDDGIGYVEDGREIFDDDLEDDALD

Please delete the paragraphs on page 7, lines 5-27 and replace them with the following paragraphs:

Sequence homology between the cloned protein ~~(piece of SEQ ID NO: 5)~~ and (a) *Vargula* luciferase ~~(SEQ ID NO: 31)~~ (b) *Renilla* LBP ~~(SEQ ID NO: 32)~~. An area of high homology in all three proteins is in bold print. **(Residues 129-186 of SEQ ID NO: 5, SEQ ID NO: 31, Residues 105-166 of SEQ ID NO: 5 and SEQ ID NO: 32, respectively, in order of appearance.)**

(a) 148

206

GTIVVT**VRVSLYD**EDNNGVMDEGKVIPSETIEDDIKDCGLLD-QDVELDYTWTQNECDL

V+VSL D + + + T+ D I D + V++ + +

YWNTWD**VKVSLRD**VESYTEVEKVTIRKQSTVVDLIVDGKQVKVGGVDVSIPYSSENTSI

353

412

(b)

105 166

S~~T~~**K**MPGTYMLMDVCATRDADDKCIEGTIVVT**VRVSLYD**EDNNGVMDEGKVIPSETIEDDIKDC

\+ TR + **VR+S+ +** N+ K I

A IKIAKLSAEKAEETRGFLRVADQLGLAPG**VRISVEE**AAVNATDSLLKMKAEEKAMAVIQSL

41 104

Three potential glycosylation sites on the protein have the consensus triplet sequence Asn-Xaa-Ser/Thr (where Xaa can be any residue except proline). Thr 216 was identified as a potential site of O-linked glycosylation by a neural network which has been trained to identify this type of glycosylation. The amino acid sequence was also entered into a neural network which had been trained to identify eukaryotic signal peptides. This

confirmed that the most likely cleavage site is between positions 20 and 21 (GSG-EE**; Residues 18-22 of SEQ ID NO: 4**).

Please delete the paragraph on page 19, line 32 to page 20, line 21 and replace it with the following paragraph:

The BOIP can also be incorporated into a defined part of a live cell by chemical means or by genetically engineering the BOIP to contain a signal peptide which locates the BOIP to the inner or outer surface of the plasma membrane or within a particular organelle such as peroxisome, mitochondrion, chloroplast, tonoplast, endoplasmic reticulum, Golgi, endosome, lysosome, secretory vesicle, nucleus, nucleolus, proteosome, or gap junction, or structure such as microtubule, cytoskeleton, nuclear skeleton, nuclear receptor, or mitotic spindle. The signal peptide, added either chemically or genetically, will normally target the normal or altered BOIP to a particular intra- or extra-cellular site for example, the sequence MLSRLSLRLLSRYLL (SEQ ID NO: 35) or part of cytochrome oxidase on the N-terminus will target the BOIP to the mitochondrion; KKSALLALMYVCPGKADKE (SEQ ID NO: 36) or MLLPVPLLLGLLGLAA (SEQ ID NO: 37) or the ER protein calreticulin at the N-terminus will target the BOIP to the endoplasmic reticulum, a KDEL (SEQ ID NO: 38) or HDEL (SEQ ID NO: 39) sequence at the C-terminus retaining it there. SKL at C-terminus targets BOIP to the peroxisome, PKKKRKV (SEQ ID NO: ~~11~~ **41**) or an extension of this SV40 large T-antigen signal will target it to the nucleus, and a palmitoylation and/or a myristoylation signal (MGCVCSSNPD (SEQ ID NO: 42) = the LCK N-terminal acylation motif from tyrosine kinase) will target it to the plasma membrane. By coupling the BOIP to another protein which targets itself to a particular site then the BOIP is also targeted here. For example, coupling the nuclear proteins nucleoplasmin or lamin B receptor to BOIP targets it

to the nucleus; cytochrome oxidase at the N-terminus targets BOIP to the mitochondria; chlorophyll at the N-terminus targets BOIP to the chloroplast; and a connexin at the N-terminus targets BOIP to the gap junction or plasma membrane, SNAP 25 to the plasma membrane.

Please delete the paragraph on page 26, lines 3-14 and replace it with the following paragraph:

Plasmid-containing apopholasin cDNA with either nucleoplasmin DNA or calreticulin DNA (with or without KDEL **(SEQ ID NO: 38)** on the C-terminus) linked to the pholasin DNA, to target the apopholasin to the nucleus or ER respectively, and the CMV promoter for expression, is transfected into HeLa cells in culture. Expression occurs within 1-3 days, and pholasin is formed by addition of the luciferin as described in Example 3. Addition of oxygen metabolites outside the cells, or hypoxic/oxygen shock generates light measured in a luminometer, showing how fast oxygen metabolites penetrate into these organelles. By imaging with a photon counting imaging camera, the number of cells permeable to oxygen metabolites can be counted. Location of the pholasin can be assessed by imaging live cells, or by using immunofluorescence with the pholasin antibody on partially-fixed cells or GFP-pholasin in live cells. Using a rainbow protein, two or more analytes can be detected together.